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Binding of Chemical Carcinogens and Mutagens to Rat Hemoglobin

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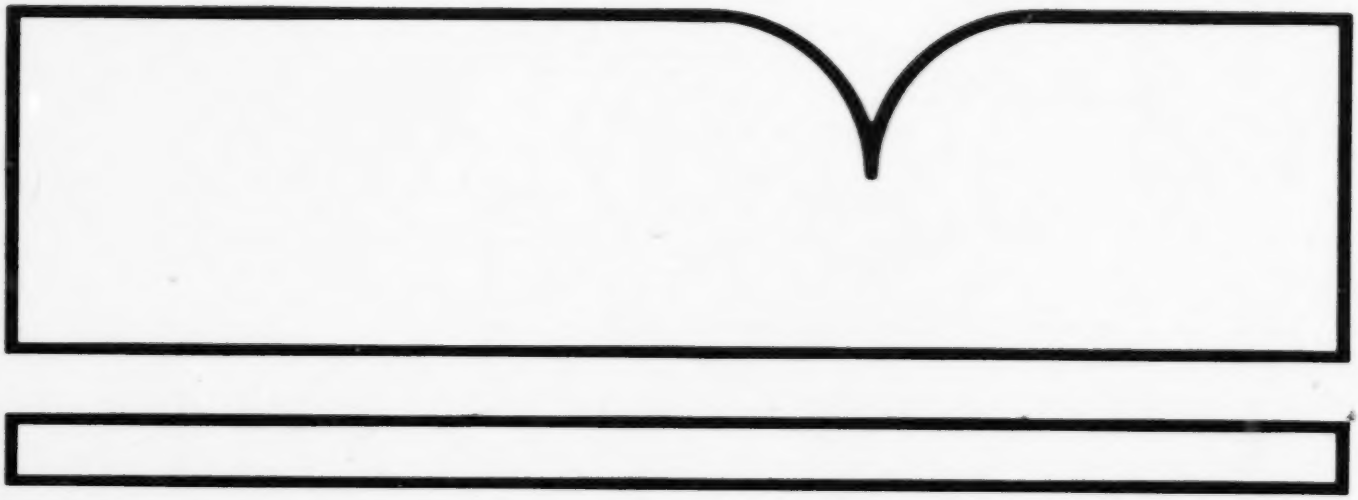

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BINDING OF CHEMICAL CARCINOGENS AND MUTAGENS TO RAT HEMOGLOBIN

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SUMMARY

The alkylation of hemoglobin is a proposed dose monitor for chemical carcinogens and mutagens. The binding of fifteen chemical carcinogens and mutagens to rat hemoglobin was determined. Direct acting carcinogens and indirect acting carcinogens including aromatic amines, halogenated hydrocarbons, nitrosamines, polycyclic aromatic hydrocarbons, aflatoxin B_1 and benzene bound hemoglobin. The efficiency of carcinogen and mutagen hemoglobin was dose dependent and ranged from 0.007 to 2.3% of an oral dose. The binding of chemical carcinogens and mutagens to hemoglobin would appear to be generic so that it could be developed into a dose monitor for a large number of known carcinogens and mutagens.

INTRODUCTION

The alkylation of hemoglobin has been proposed as a dose monitor for chemical carcinogens and mutagens [1]. The mechanism of action of most carcinogens and mutagens involves their covalent binding to nucleophilic sites in cellular macromolecules. The advantages of the hemoglobin binding assay as a dose monitor are (1) that blood is readily and repeatably obtainable from people and laboratory animals, (2) large quantities of hemoglobin are present in the blood (approx. 10—15% by weight) and (3) the stability of circulating hemoglobin which is directly dependent upon red cell half-life and is on the order of months. The stability of circulating carcinogens bound to hemoglobin could allow (1) the monitoring of an exposure long after it has ceased and (2) the accumulation of damage produced by low chronic exposures that by themselves are not detected.

A limited number of chemical carcinogens and mutagens have been shown to covalently bind hemoglobin. These chemicals include methyl methanesulfonate [2], ethylene oxide [3,5], vinyl chloride [4], and

dimethylnitrosamine [5]. In this communication, we report this binding for a number of different chemical classes of carcinogens and mutagens. On this basis we suggest the application of monitoring their binding to hemoglobin as an estimate of dose.

MATERIALS AND METHODS

Animals. Male Sprague—Dawley rats (Charles River Co.) weighing 200—250 g were used in these studies. They were fed Purina laboratory chow (Ralston Purina Co.) and given distilled water ad libitum.

Radiolabeled chemicals. Carbon-14 radiolabeled chemicals were used in these studies. Radiochemical purity was checked immediately prior to use with a Spectra Physics 8000 High Performance Liquid Chromatograph (Spectra Physics, Inc.) equipped with an ODS column (Spectra Physics, Inc.) and eluted with a water:methanol gradient. All radiolabeled chemicals were judged to meet the manufacturer's specifications and to exceed 95% purity. Benzo[*a*]pyrene, methyl methanesulfonate, and naphthalene were purchased from Amersham Corp., Arlington Heights, IL; nitrosodiethylamine, diethylbenz[*a*]anthracene, nitrosodimethylamine, methyl-*N*-nitrosourea, ethyl-*N*-nitrosourea, acetyl-2-aminofluorene, benzene, benzidine, 3-methylcholanthrene, carbon tetrachloride and methyl-*N*-nitroso-*N*-nitrosoguanidine from New England Nuclear, Boston, MA; aniline, chloroform and phenol from ICN Pharmaceuticals, Inc., Irvine, CA and aflatoxin B_1 from Moravek Biochemicals, City of Industry, CA.

Experimental design. The chemical of test was administered intragastrically (1 ml/kg body wt.) and blood was collected 24 h later under ether anesthesia by cardiopuncture with a heparinized syringe. The erythrocytes were collected by centrifugation and washed twice with heparinized saline. They were lysed with 4-fold volume of distilled water by vortexing and incubating for 15 min. The pH was adjusted to 5.8 with hydrochloric acid and the cellular debris removed by centrifugation.

The heme and unbound chemical was removed by a modification of the procedure of Arson and Mirsky [6]. A 0.3-ml aliquot containing 20—30 mg hemoglobin was diluted with water to 3 ml and slowly added to 30 ml of cold 1% HCl in acetone while vortexing. The protein was collected by centrifugation and washed sequentially with 1% HCl in acetone and acetone. The protein was dissolved in 0.3 ml water at 60°C. To this, 0.7 ml Protosol (New England Nuclear, Boston, MA): ethanol mixture (1 : 2, v/v) was added and incubated in a sealed scintillation vial at 60°C with shaking until clear (1 h). Tert butyl hydroperoxide (0.5 ml) was added, the vials sealed and incubated at 60°C with shaking until decolorized. After cooling, 15 ml Bio Fluor (New England Nuclear) and 0.5 N HCl were added. The radioactivity was measured in a Beckman 8100 liquid scintillation counter (Beckman Instrument Corp.) with counting efficiency of 65—70%.

Hemoglobin concentration was determined prior to the removal of the heme. Cyanohemoglobin was formed with Zap-Oglobin II (Coulter

TABLE I

COVALENT BINDING OF CARBON — 14C RADIOLABELED CARCINOGENS AND MUTAGENS TO HEMOGLOBIN

Results are mean values ± S.E.M. for 6 animals in each group. Column *A*, pmol/g Hb; column *B*, pmol/g Hb/μmol dose. Chemical dose is expressed as μmol/kg.

Chemical dose	Amount bound		
	A	*B*	Average
A. Carcinogens			
(1) Methyl methanesulfonate			
1	3264 ± 411	3264	3320
0.1	338 ± 27	3380	
(2) Dimethylnitrosamine			
1	575 ± 49	575	697
0.1	81.9 ± 13.3	819	
(3) *N*-nitrosomethylurea			
1	308 ± 31	308	302
0.1	29.5 ± 2.7	295	
(4) *N*-Nitroso-*N*-Ethylurea			
1	203 ± 9.5	203	217
0.1	23.2 ± 1.9	232	
(5) Benzo[*a*]pyrene			
10	1930 ± 608	193	181
1	114 ± 28	114	
0.1	28.4 ± 10.6	284	
0.01	1.35 ± 0.12	135	
(6) Methyl-*N'*-nitro-*N*-nitrosoguanidine			
1	193 ± 9.4	193	180
0.1	16.6 ± 2.9	166	
(7) 3-Methylcholanthrene			
1	117 ± 11.7	117	165
0.1	21.2 ± 0.9	212	
(8) Nitrosodiethylamine			
1	153 ± 6.6	153	153
(9) Benzene			
3	465 ± 86	155	144
1	125 ± 14	127	
0.1	15.0 ± 1.4	150	
(10) 2-Acetylaminofluorene			
1	85.1 ± 9.8	85.1	108
0.1	13.1 ± 0.9	131	
(11) 7,12-Dimethylbenz[*a*]anthracene			
1	99.8 ± 23.7	99.8	99.8
(12) Aflatoxin B_1			
1	47.3 ± 2.1	47.3	39.4
0.1	3.74 ± 0.63	31.4	
(13) Aniline			
4.8	118 ± 11.1	24.8	24.8
(14) Benzidine			
1	22.5 ± 3.6	22.5	22.5
(15 Chloroform			
10	136 ± 15	13.6	14.2
1	14.9 ± 1.1	14.9	
(16) Carbon tetrachloride			
10	102 ± 12.5	10.2	10.2
B. Non-carcinogens			
(1) Naphthalene			
26	729 ± 33.2	28.0	28.0
(2) Phenol			
9.4	2.74 ± 1.35	0.3	0.3

Electronics Corp.) and the concentration determined with a Coulter Hemoglobinometer (Coulter Electronics Corp.).

RESULTS

The binding of sixteen chemical carcinogens and mutagens and two non-carcinogens to rat hemoglobin was determined. The results in Table I are presented in order of decreasing relative amounts bound. The ability of chemical carcinogens and mutagens to bind hemoglobin varied from 10.2 to 3322 pmol/g hemoglobin/μmol of oral dose for carbon tetrachloride and methyl methanesulfonate respectively. Phenol, a non-carcinogenic metabolic of benzene, did not bind hemoglobin. The non-carcinogenic polycyclic aromatic hydrocarbon, naphthalene, did bind hemoglobin; however the extent of binding was much lower when compared to the carcinogenic polycyclic aromatic hydrocarbons. Naphthalene has previously been demonstrated to bind protein and DNA [7].

The binding of chemical carcinogens and mutagens to hemoglobin was dose dependent. Of those compounds analysed, the efficiencies of hemoglobin binding of 0.1 and 1 μmol/kg oral dose of a chemical were not significantly different. This would indicate for the eleven chemicals where the binding of at least two doses was determined, that the binding to hemoglobin was dependent upon dose. Assuming 50 ml blood/kg body wt. and 14 g Hb/100 ml blood, a dose of 1 μmol/kg body wt. would result in 0.07—23 nmol/kg body wt. bound to hemoglobin. Thus, the efficiency of hemoglobin binding for chemical carcinogens and mutagens varied between 0.007% and 2.3% of an oral dose.

DISCUSSION

The alkylation of hemoglobin by chemical carcinogens and mutagens has been proposed as a dose monitor [1]. We plan to employ the binding of chemical carcinogens and mutagens to hemoglobin as an estimate of dose resulting from environmental exposure in laboratory animals and epidemiological studies. As a preliminary to these studies we determined whether the binding to hemoglobin was a generic effect of chemical carcinogens and mutagens.

Previously published results have demonstrated that alkylating agents covalently bind hemoglobin. We have determined that a broad spectrum of chemical classes of direct and indirect acting carcinogens and mutagens bind hemoglobin. Polycyclic aromatic hydrocarbons, aromatic amines, nitrosamines, halogenated hydrocarbons, aflatoxin B, benzene and direct alkylating agents bound hemoglobin. These classes of chemicals represent the major chemical classes of carcinogens and mutagens. The application of analytical techniques including amino acid analysis and gas chromatography—mass spectrometry to the identification and estimation of the extent of carcinogen bound to hemoglobin [1,8] should result in a dose monitor applicable for most carcinogens and mutagens.

Both direct and indirect acting carcinogens and mutagens bound hemoglobin. Methyl methanesulfonate, a direct acting mutagen, at 3320 pmol/g Hb/μmol dose was bound to the greatest extent. Methyl methanesulfonate is a very weak carcinogen. This was followed by the strong and indirect acting carcinogen dimethylnitrosamine. Dimethylnitrosamine bound hemoglobin to an extent greater than the two other direct acting carcinogens *N*-nitrosomethylurea and *N*-nitroso-*N*-ethylurea. The comparison of the extent of hemoglobin binding to carcinogenic activity would appear to be possible only within chemical classes.

The binding to hemoglobin of chemical carcinogens and mutagens was dose dependent. The efficiency of hemoglobin binding resulting from doses that differed by an order of magnitude was similar. The high efficiency of binding at the low dose 0.1 μmol/kg would indicate that environmental levels of certain chemical carcinogens and mutagens would be sufficient to allow the use of hemoglobin binding for estimating exposure in laboratory animal and epidemiological studies.

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